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# SOME OBSERVATIONS

ON

# THE STRUCTURE AND DEVELOPMENT

OF

# VOLVOX GLOBATOR,

## AND ITS RELATIONS TO OTHER UNICELLULAR PLANTS.

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(Read May 26, 1852.)

Three forms, or, as they are commonly regarded, species of *Volvox* are described and figured in Ehrenberg's great work, and have been noticed by other observers. These are *V. globator*, *V. aureus*, and *V. stellatus*. A fourth very similar organism has also been described under the name of *Sphærosira Volvox*.

As I regard the three first named of these at all events, merely as forms or phases of one and the same species, the following observations will apply in some respects to all of them. They have more particular reference, however, to the common form of *V. globator*, which happens to be that most accessible to me.

This beautiful and well known object, which was first noticed by Leeuwenhoek, received little satisfactory elucidation until it fell under the observation of Ehrenberg, whose account of its structure and notions respecting its nature have been adopted by most subsequent observers, and have been received with little opposition until very lately—in fact until the beginning of last year. At that time Professor Williamson read a paper on the subject before the Manchester Literary and Philosophical Society, which is published in the 9th volume of their Memoirs.

Professor Williamson's observations have led him to conclusions in many points opposed to those arrived at by Ehrenberg, and especially are they confirmatory of Siebold's original view of the vegetable nature of *Volvox*. With respect to some points of structure, however, concerning which Professor Williamson differs from the Prussian observer, I am inclined, from my own observations, to side with the latter, whose errors in the case of *Volvox* are not those of direct observation; but in this instance, as in very many others, it is obvious that Ehrenberg has allowed his imagination, working upon preconceived notions, to play the part of reason in the interpretation of correctly-observed phenomena; he has thence, in the explanation of what he has *seen* correctly, fallen occasionally into great and important errors. Whilst it cannot be denied that the recent progress of knowledge with respect to the structure and nature of the lowest classes of organised beings, places the observer of the present day in a position so much more advantageous, that it is scarcely fair to institute a comparison between him and the great and laborious Prussian microscopist, at the time his principal works were written, still it is much to be regretted that these modern lights, clear as they are, have not apparently been allowed to penetrate his mind, and that one to whom science is so much and so deeply indebted should retain views long since deservedly exploded by nearly all competent observers.

The more common and best known form of *Volvox globator*, to the naked eye, or under a low power, appears as a transparent sphere, the surface of which is studded with numerous, regularly placed green granules or particles, and which contains in the interior several green globules, of various sizes in different individuals, though nearly always of uniform size in one and the same parent globe.

These internal globes, which are the young or embryo *Volvox*, at first adhere to the wall of the parent cell, although the precise mode of connexion is not very apparent. When thus affixed, they are in a different concentric plane to the smaller green granules. At a later period, and after they have attained a certain degree of development, these internal globes become detached, and frequently exhibit a rotatory motion, similar to that of the parent globe.

In the form of *Volvox*, termed *V. aureus* by Ehrenberg, the outer sphere, or cell, exhibits precisely the same structure as the above, the only apparent difference between them consisting in the deeper green colour of the internal globules. These, however, soon exhibit a more important distinctive character in the formation of a distinct cell-wall of considerable thickness around the dark green globular mass. This wall becomes more and more distinct; and, after a time, the contents, from dark green, change into a deep orange-yellow;

and simultaneously with this change of colour the wall of the globule acquires increased thickness, and appears double.

The third form, or *Volvox stellatus*, differs in no respect from the two former, except in the form of the internal globules, which exhibit a stellate aspect, caused by the projection on their surface of numerous conical eminences, formed of the hyaline substance, of which the outer wall of the globule is constituted. The deep green colour of the contents of these stellate embryos, and their subsequent changes into an orange colour, at once point out their close analogy with those of *V. aureus*. I have no doubt of their being merely modifications of the latter; and, in fact, the two forms are very frequently to be met with intermixed, and on several occasions I have observed smooth and stellate globules in the interior of one and the same parent globe.

The organism described and figured by Ehrenberg, under the name of *Sphærosira volvox*, also presents the appearance of a transparent globe set with green spots, but it differs from the foregoing in two important respects.

1. In the absence of any internal globules or embryos.

2. In the irregular size of the green granules lining the wall, which, instead of being of a uniform size, are of various dimensions (fig. 13, Pl. V.). The different sized granules are irregularly disposed, although, in relation to the sphere itself, they, or rather the centres of them, are as regularly distributed as in the three just-described forms. What is rather remarkable with respect to this form is the circumstance, that the larger granules are not disposed over the whole periphery of the sphere, rarely occupying more than two-thirds of it, towards one side. In the more minute description of the elements of the above-mentioned organisms, the investigation of which requires the higher powers of the microscope, it will be convenient to commence with the common *Volvox globator;* and as the tracing of the development of the internal embryonic globules affords the readiest road to a comprehension of the true structure of the mature globe, I shall proceed in that course.

The internal embryonic globules are visible in the young *Volvox* while still within the parent; but as they are at first concealed by the density of the wall of the young *Volvox*, the very earliest stage of formation of the embryo cannot be readily noticed. In the earliest state in which these bodies can be observed, they appear as a globular, or rather discoid, nucleated cell (fig. 3), which, besides its apparent central nucleus, contains a number of minute spherules placed towards the periphery. At this time no distinct wall can be detected, the whole embryo (to use a convenient though incorrect term) apparently consisting of a homogeneous substance, with a lighter nuclear-looking space in the centre, and the above-mentioned spherules towards the periphery. This nucleated cell, as it may be termed, although without a cell-wall, increases in size, and the solid or coloured contents appear to retreat from the centre, which becomes clearer and clearer towards the periphery, which gradually becomes more and more opaque. As the cell grows, the nucleus (?) seems to disappear, or to be converted into the clear central space; or, it may be, broken up and confounded in the more opaque contents. The number of spherules increases as the cell grows, and it is very soon apparent that the now very thick parietal deposit of cell contents is breaking up into small portions or lobular masses, the centre becoming clear, and apparently filled only with a clear aqueous fluid. When the cell has thus acquired a considerable size, the contents begin to undergo segmentation, as pointed out in the case of *Volvox*—I believe first by Professor Williamson. This process commences and proceeds precisely as in the ova of animals—the contents dividing first into two, and then each of the halves into two, and so on, till the division becomes too minute to allow of the counting of the segments. It is to be remarked, moreover—and I think this has not been noticed before—that the bright spherical bodies multiply quite as rapidly, if not in a more rapid ratio, up to a certain point, than the segmentation goes on, so that each segment of the still-dividing mass always exhibits two, three, four, or even more of these particles (figs. 1, 2). Ultimately the segmentation ends in the formation of innumerable green bodies, which are closely packed round the periphery of the cell. These bodies, though perfectly defined, are not at first separated by any clear space, and each contains at least one of the bright spherules alluded to (fig. 3). By their mutual pressure, these soft corpuscles of course assume an hexagonal figure, and they are now about 1-4000th of an inch in diameter, or rather more. As soon as, or even before, the segmentation commences, a distinct though delicate membrane, surrounding the embryonic mass, is quite evident, as described by Mr. Williamson; and beyond this is usually to be observed a very delicate zone of apparently gelatinous matter, which is sometimes so delicate as to escape observation, but may, I believe, always be detected by the use of a solution of iodine.

When the segmentation is completed, in the way above described, the embryo *Volvox* exhibits the appearance of a spherical body composed of a transparent membrane lined with distinct, uniform-sized, contiguous hexagonal masses. It continues to grow, and very soon clear lines become apparent between the green masses, which are thus very distinctly defined, retaining the same hexagonal form—each with an apparent nucleus, which is probably derived from the bright spherule contained in it, but as yet without brown spot, clear space (vacuole), or vibratile cilia. As the embryo continues to grow, the spaces between the green masses continue to increase; the green bodies gradually lose the hexagonal form, and assume the appearance of the ciliated zoospore next to be described. They are now about 1-3000th of an inch, or thereabouts, in diameter, and the embryo, detached from its parent, becomes a free *Volvox* in its interior. We have thus arrived at the complete *Volvox*, and from the mode of its formation it is apparent that it consists of a transparent wall lined with the green bodies, and hollow in the interior; and also that it is surrounded, at all events while within the parent, with a delicate transparent areola, apparently of gelatinous matter We have now to examine more minutely the structure and nature of the green granules, and the further changes they undergo.

Upon examination of the wall of a full-grown *V. globator* with a sufficient magnifying power, it will be seen, upon viewing the edges, as it were, of the image in the field, with the object so arranged as to bring the equatorial plane exactly into focus (fig. 5), that the green granules are, in fact, vesicular or semivesicular bodies of a flask-like or conical form, about 1-3000th of an inch in transverse diameter, and placed at uniform distances apart. Each of them is prolonged outwardly into a sort of peak or proboscis of a transparent and colourless or hyaline material, and from which proceed two very long vibratile cilia, which in close contact at first, pass through the parent cell-wall, upon the outer side of which they separate widely and perform very active movements. The outer cell-wall presents a minute infundibuliform depression at the point of exit of the cilia. It will also be observed, that each ciliated cell or zoospore, as it may analogically be termed, contains a green granular mass or masses, composed, for the most part probably, of chlorophyll granules and a more transparent body, which I suppose may be regarded as a nucleus, and derived, as it would appear, from one of the bright spherules which have been noticed before. At an early period after the maturity or completion of the zoospores they exhibit a minute, circular, clear space, or sometimes, but I think rarely, more than one, which is worthy of very attentive consideration. This space is of pretty uniform size in all cases, and about 1-9000th of an inch in diameter. It may be situated in any part of the zoospore, or not unfrequently in the base, or even in the midst of one or other of the bands of protoplasm connecting it with its neighbours. Its most important character consists in its contractility—a property already known to be possessed by similar spaces or vacuoles in vegetable spores. But what appears to me a very curious, and as yet unnoticed peculiarity of this contraction, consists in the fact that it is very regularly rhythmical. In several cases in which I have watched the phenomenon in question, uninterruptedly, for some time, the contractions or pulsations occurred very regularly at intervals of about 38″ to 41″. In one case, however, if I was not misled in the observation, the interval was about twice this, viz., 1′ 25″. The contraction, which appears to amount to complete obliteration of the cavity of the "vacuole," takes place rapidly or suddenly, as it were, whilst the dilatation is slow and gradual. The interval above noted was measured between one sudden contraction and the next, and about half of it perhaps was taken up by the slow dilatation of the space. This contractile vacuole always reappears in precisely the same spot. It would seem to exist, or at all events to present a contractile property only for a limited period, and to disappear soon after the formation of the brown spot, when, as I conceive, the zoospore has reached its maturity. The most favourable cases in which this contractile space is to be sought for, are those in which the *Volvox* is in the most vigorous state, and especially in that variety in which, owing perhaps to the copious supply of nutritive matter, the amount of protoplasm is very abundant, and the zoospores consequently very numerous and connected to each other, not by slender filaments but by wide processes, as in figs. 26, 27, which latter shows a contractile space situated in the base of one of the connecting bands of protoplasm. With the exception of the small space occupied by this contractile spot, the zoospore at first appears to be quite solid, and no distinct wall can be perceived around the green matter, but it rapidly changes. Owing either to the expansion of the vacuole, above described, after it has lost its contractile property, or to the formation of others of a different nature, and also perhaps in some degree to the absorption or consumption of some of the colouring contents, the zoospore gradually becomes more and more transparent (fig. 7); till at last, the greater part of it is clear and colourless, and what remains of the green matter contracts into a small irregular mass, adherent to the bottom or sides of what is now a cell—primordial cell of Cohn. (Figs. 5, 6.)

Each cell, when fully formed, usually presents a brown spot, which is adherent to one side of the cell towards the narrow end (figs. 5, 6); and what is remarkable, it will be noticed in a perfect specimen, that the brown spots are placed in a corresponding situation in all the cells, that is to say, all the cells appear to look the same way. This is the so-termed eye-spot of Ehrenberg. When examined with a high power (800—900 diam.) it presents the form of a cup or disc, concave on the side which looks outward, and convex on the other. Though placed quite on the side of the cell, and projecting a little upon it, the brown spot is nevertheless always covered by a thin membranous expansion of protoplasm, or, in other words, it is always lodged within the substance of the zoospore. Though most usually present, the brown spot does not appear, in all cases, to be at any period a necessary constituent of the zoospore. It is one of the most persistent however, remaining visible as long as any portion of the zoospore is discernible. Besides the above-described elements, the zoospore, when viewed from above, exhibits two highly refractive spots placed side by side, which seem to represent the insertions or origins of the two vibratile cilia.

The periphery of the cell presents a clear line, and appears to be formed of a delicate membrane—although, in the earlier stages of the existence of the zoospore, that is, before the formation of the eye-spot, or disappearance of the contractile vacuole, the whole evidently consists of a homogeneous substance, in which the above described parts are imbedded. From the periphery of the zoospore proceed six thread-like processes, connecting it with as many of its neighbours. These threads appear to be simply continuations of the *quasi* cell-wall, and to be of the same nature chemically as it, as are also the vibratile cilia. The connecting threads are sometimes double, or even triple, between some one or more of the surrounding cells, and they are invariably continuous between the two cells.

This description applies more particularly to the zoospores *in situ.* When the *Volvox* is ruptured, many appear to become immediately detached, and to be washed out, as it were, with the aqueous contents of the parent cell. Under these circumstances they lose some of their previous regularity of form, but not much; they become more globular and the beak less prominent, but in other respects they appear much the same as before. The two vibratile cilia remain in connexion with them, and continue their active movements. This is opposed to Mr. Williamson's statement, that " when thus liberated they exhibit no traces of the two cilia, or proboscides" of Ehrenberg, and agrees with that of the latter.

Among the thus liberated ciliated zoospores will usually be found numerous detached cilia, which, as is observed by Mr. Williamson, are generally more or less coiled at one end into a ring. And besides these I have not unfrequently noticed some extremely delicate annular bodies, about 1-9000th of an inch in diameter, perfectly clear and colourless, which seem as if they had escaped from the interior of the ruptured zoospore: but of this and their true nature I am unable to speak positively.

Having thus described what I conceive to be the anatomy of the common form of *Volvox globator*, I will thus sum up the result of what my observations have led me to conclude as to its structure.

1. That it originates in an apparently nucleated, discoid cell, which is generated in the interior of the parent, and liberated in a perfect, though not fully matured form; within which are contained similar germs.

2. That the contents of this apparently nucleated discoid cell, consisting of a grumous material and refractive amylaceous (?) spherules, after a time undergo segmentation, at the same time exhibiting a distinct wall, beyond which is a delicate areola, apparently of a gelatinous consistence.

3. That this segmentation, attended with a corresponding augmentation in the number of the refractive spherules, terminates ultimately in the formation of numerous contiguous particles or segments.

4. That these ultimate segments are gradually separated from each other, remaining connected only by elongated processes or filaments, and constituting the ciliated zoospores of the mature *Volvox*.

5. That these zoospores at first are simple masses of protoplasm, containing a transparent nuclear body, and that afterwards they present for a time clear, circular spaces, which contract rhythmically at regular intervals; and are subsequently furnished with a brown eye-spot; and at a very early period with two long retractile cilia, which, arising from an elongated hyaline beak, penetrate the parent cell-wall, and exert active movements external to it.

6. That in a concentric plane internal to these ciliated zoospores are placed the germs of future individuals destined to follow the same course.

Having thus traced one form of *Volvox* through its course of development, I will proceed much more briefly to the others.

In *V. aureus*, as I have said, the constitution of the wall of the parent cell is exactly as above described. At its earliest appearance also the internal embryonic body cannot be distinguished from that of the ordinary form, except in its deeper green colour. It afterwards, however, acquires a thick wall, changes its colour to yellow without material alteration in size, and acquires a second equally firm and distinct envelope, or rather, as I believe, the original contents contract somewhat, and then form a second coat around themselves. Eventually a considerable space exists between these two coats (figs. 10, 12), which space is occupied by a clear and apparently aqueous fluid, but upon the addition of a solution of iodine a granular cloudiness is produced in this fluid. The contents of the inner cell consist chiefly of amylaceous grains mixed with a greenish material in the one case, and with a bright yellow apparently oily fluid in the other. The amylaceous particles are of an irregular botryoidal form, and far from uniform in size. As regards the future destination of this form of germ, I am as yet in total ignorance; there can, however, I think, be little doubt but that it represents the "still" form of spore of other Algæ—that it may, in fact, be termed the "winter spore" of *Volvox*, destined, owing to its more persistent vitality, to continue the species, when its course of development in the usual way is interrupted by surrounding circumstances.

Of that form of *Volvox* termed *V. stellatus*, I would only here observe that it seems to me merely a modification of the one last described, and that it appears to follow the same course of change and doubtless of future development.

With respect to *Sphærosira volvox*, my observations have been very limited, and I by no means desire to express myself with certainty as to its relationship to the forms above described. I merely surmise that it may be found to represent a peculiar mode of development of one and the same species.

In external aspect, except in the want of uniformity in the size of the ciliated zoospores, it appears to agree in all respects with *V. globator*. It however contains no internal embryonic bodies, and it is therefore only to the ciliated cells that any reference need here be made. The smaller ones appear to me to resemble in all respects those of *Volvox globator*, and each to possess two cilia, which is important if true, because the only distinction between *Volvox* and *Sphærosira* in Ehrenberg's classification depends upon the circumstance, that in *Sphærosira* there is only a single cilium to each zoospore, whilst there are two in *Volvox*.

My supposition that *Sphærosira volvox* and *V. globator* are allied, is founded, it must be owned, not upon any direct observation, but chiefly upon the fact, that in the water in which the specimens of *Volvox* I had under examination were contained, there was at first none of the *Sphærosira* any more than of *V. aureus* observable, and that after some days both were very numerous.

The difference I am about to describe in the after development of the ciliated zoospores, is not by any means a sufficient ground upon which they should be deemed distinct species, because much greater differences are known to exist in other of the lower Algæ during their various forms of development without its being thence allowable to suppose that they are of different species.

In *Volvox sphærosira* then, as at all events it may be termed, the larger green granules are in fact the ciliated zoospores in a stage of further progressive development. In the same specimen they will be seen in all stages of division or segmentation (fig. 13)—first into two, then into four, and so on till, as in the case of the embryo *Volvox*, the ultimate result of the segmentation constitutes numerous minute ciliated cells or bodies (fig. 14)—not, however, as in that case, lining the inner surface of the wall of a spherical case, but forming by their aggregation, a discoid body, in which the separate fusiform cells are connected together at one end, and at the other are free, and furnished each with a single cilium. In this stage these compound masses become free and swim about in the water, constituting, in fact, a species of the genus *Uvella*, or of *Syncrypta* of Ehrenberg.

With respect to the chemical constitution of the above described parts, the following are the results at which I have arrived:—1. By the use of iodine and sulphuric acid, tried repeatedly and in various ways, I have never succeeded satisfactorily in eliciting any tinge of blue in the wall of the mature *Volvox*. I therefore conclude that it contains no cellulose. It is invariably coloured, by the above re-agents, of a deep brown colour, and when thus coloured, this outer wall presents no trace whatever of structure; it appears uniformly transparent and homogeneous. The ciliated zoospores, also, with the connecting filaments and cilia, are turned brown, but of a very deep brown, by the same re-agents, excepting usually one or more particles in the interior of each, which are apparently turned blue. I am not satisfied as to the chemical re-action of the brown spot; it appears to assume a blue colour, but from the intensity of its colour and consequent opacity I am not sure that this is the case.

The embryo cell, when young, is turned a deep brown, but when older and fully formed, but before it has arrived at maturity, it will be found that it is only the green masses, or future ciliated zoospores, that are thus changed, the cell-wall acquiring scarcely any tinge of brown. But when a young cell thus tested with iodine and sulphuric acid is ruptured, I have occasionally noticed that the fluid contents contain an abundance of minute bluish flocculi—I use the word flocculi because the particles are light and flocculent, and not at all like any of the more ordinary and more solid forms of amylaceous matter. The quantity of this flocculent matter appears to be greater towards the periphery of the cell, and, in fact, it would seem that the green bodies are at this time imbedded as it were in an amylaceous matrix, which they not improbably assimilate, because in the mature cell nothing of the sort is apparent.

In the embryonic bodies, however, or winter spores of *V. aureus* and *stellatus*, the presence of cellulose is rendered abundantly evident in the two coats forming the tunic of the spore by the blue colour produced in them by iodine and sulphuric acid (fig. 11); nearly as distinctly, in fact, as it is in the tunic of *Micrasterias* and other *Desmidieæ.* The apparently clear fluid between the two tunics is rendered brownish and turbid at the same time (figs. 11, 12), and the solid contents of the interior are shown to consist, for the most part, of amylaceous grains of the peculiar botryoidal form above noticed. (Fig. 9.) The yellow oil-like fluid in the ripe spore acquires a green tint under the action of the same re-agents. (Fig. 9.)

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## Appendix.—(*October*, 1852.)

The above are the observations read at the Microscopical Society. I am now satisfied that they afford an account of but one of the multiform varieties under which *Volvox* occurs at different times and places. I must own also, that at the time my observations there detailed were made, I was unable to reconcile much of what I saw with some of the statements and figures in my friend Professor Williamson's ingenious paper on the same subject. Subsequent investigation, however, and some correspondence with him, have satisfied me that I was hasty in drawing conclusions from one form only of a very protean object. I freely confess, that in much, in respect to which I had conceived Professor Williamson had fallen into some error of observation, he has been quite right, though at the same time I must say that his explanations of the appearances described and figured by him, do not exactly accord with my notions respecting them. I still maintain that the structure of the wall of *Volvox*—upon which alone I think we are disagreed—is essentially such as I have described it, viz., that it is formed by a continuous, external tunic, lined by the ciliated zoospores. Professor Williamson, on the other hand, as I understand him, conceives the globe to be "a hollow vesicle, the walls of which consist of numerous angular cells filled with green endochrome, &c., the intercellular spaces being more or less transparent." The ciliated zoospore, therefore, according to him, is not a mass of vegetable protoplasm, without distinct wall, and precisely analogous to a *Euglena*, or other naked zoospores, but represents the endochrome of a cell having two walls, an external and an internal, which latter is "a ductile cell-membrane, lining the interior of each cell and surrounding the cell-contents," and which "inner membrane becomes separated from the outer cell-wall excepting at a few points, where it is retained in contact." And he thus explains the mode of formation of the connecting filaments. In this case, therefore, these filaments would never pass directly from one green mass to another, but would of course be interrupted in their course by the walls of two contiguous cells. That this, however, is not the case in the form of *Volvox*, which formed the subject of my paper, is sufficiently obvious. But it is nevertheless true, as I find from examination of Professor Williamson's specimens, that his representation is, in certain cases, equally correct, as I shall afterwards explain. Another circumstance also noticed by Mr. Williamson, and which, till he pointed it out particularly to me, had, though not altogether unnoticed, been disregarded, is the existence of delicate lines between the green granules, and dividing the wall of the *Volvox* into very regular hexagonal spaces, in the centre of each of which is placed one of the green granules. The former of these conditions—which, though I have never met with it myself distinctly in specimens from any other locality, seems to be sufficiently abundant in the neighbourhood of Manchester—is represented in figs. 15, 16, 17, 18, 19, 20. In these it will be seen that the central green body is surrounded, at variable distances, by a tolerably thick, distinct membrane or wall, and that numerous irregular filaments, where they exist at all, extend from the central mass to this wall, and there terminate, and do not pass from one green mass to another, as in the usual form. Now, I explain the way in which the zoospore is thus modified, in this way: I regard the external membrane merely as the boundary-wall of the original zoospore, and, like the entire body, as composed of vegetable protoplasm; and I believe that this peculiar appearance is produced by a great and unusual expansion of the interior of the zoospore (by endosmosis of fluid probably), by which the outer or peripheral layer is separated from the remainder and principal part of the mass, containing the chlorophyll and nucleus, or supposed nucleus, &c. Zoospores, in fact, in this condition might be said to be dropsical. That this separation of the wall from the contents arises in this way, and not, as Mr. Williamson says, from the shrinking of contents, is, I think, sufficiently obvious from several considerations, and is rendered very clear, if we trace the progressive stages of the hydropical enlargement in one and the same *Volvox*, as I have done in the figures above cited.

In this series it is easy to observe the earliest formation of the clear space up to the most extreme dilatation of which the cell is capable, owing to its contiguity with others. Of course when a number of cells are thus enlarged and mutually compressed, they assume an hexagonal form ; but this hexagonal arrangement must not, as it appears to me, be confounded with another, to which I have before alluded, and which I conceive to be due to a different circumstance altogether. In fig. 15 of this series, some cells will be obseved little if at all altered, from what I assume to be the normal form, and it will be seen that these little altered cells are mutually connected by the usual continuous filaments. In fig. 16 the zoospores are more expanded, and being in contact in many points, the connecting threads are absent; fig. 17 shows a further degree of expansion, but more irregular, and with irregular connecting filaments. In fig. 18 the enlargement is nearly as great as it can be, and numerous threads or processes of protoplasma extend from the central mass to the wall, just as they do in almost any vegetable cell from the nucleus to the primordial utricle, which utricle, in fact, is represented by the cell-wall in the case we are discussing. In fig. 19, the dilatation is complete, and, owing to the greater age of the specimen from which this figure was taken, the protoplasma is much wasted, and all the filamentary processes completely gone. A faint granular appearance occupies the cavity of the primordial cell. It is a curious fact, as showing perhaps that all the vital action in the cell resides in or around the nuclear mass, that not unfrequently the central mass after considerable expansion of the cell, and the formation in that way of one wall, will begin to throw off a second. This condition is represented in a more highly magnified drawing in fig. 20.

Although I have not myself seen any natural specimens in which this condition of the zoospores was present, except those for which I have been indebted to Mr. Williamson, still I have repeatedly observed a partial appearance of the same kind to take place, when a specimen of *Volvox* of the normal sort is kept for some hours under observation in the microscope. Figs. 21, 22, 23 show the series of changes that took place in a certain number of zoospores watched at intervals, and left undisturbed for about twenty-four hours. (Fig. 21, 10 A.M., Oct. 4. Fig. 22, 1 P.M., Oct. 4. Fig. 22, 8 A.M., Oct. 5.)

Now with respect to the other form of hexagonal areolation, for my knowledge of which, as I have stated, I am chiefly indebted to Professor Williamson, and which is represented in figs. 24, 25, I have already observed, that I regard it as quite distinct from that produced by the mutual pressure of contiguous dilated zoospores. Professor Williamson appears, from what he has told me by letter, to consider that this appearance is invariably present, or at all events that it can be elicited in all cases by appropriate means. I must confess however, that I have not been successful in seeing it, or in producing it in very many instances, and that I believe it is occasionally impossible by any means to demonstrate its existence. At some periods not a single specimen from a given locality will exhibit it, whilst at another, every individual will show it at the first glance. Thus in the month of August last, when, in a certain pond on Blackheath there was the most incredible abundance of *Volvox*, so great in fact as to render the water at the lee side of the pond in certain spots of a deep green colour, and to cause it to afford, when collected, a very strong herbaceous or confervoid smell, the majority of the plants exhibited the stellate form of spores, or rapidly acquired spores of that character, and very many were in, or soon assumed, the form of *V. aureus*. They seemed in fact to be entering upon their hybernating state. Many among them, however, though all small and starved-looking, were of the common kind; in all these Mr. Williamson's hexagonal areolation was very distinct. In the month of October, however, upon returning to the same pond, I was able to find very few *Volvoces* at all, and all of the usual kind; in none of these could I detect the least appearance of the same arrangement. I therefore conclude that the greater or less distinctness, or complete absence of this character, is to be referred to external conditions with which we are not fully acquainted. The appearance itself I explain in this way. It appears to me that each zoospore is imbedded in a distinct gelatinous or semi-fluid envelope of considerable thickness, and that the hexagonal areas are formed by the sides of these distinct masses of gelatinous matter coming into contact. I am inclined to think that there is no distinct membrane containing this gelatinous matter: if there be, it must be infinitely thin, because the line of contact is extremely delicate and single. I conceive, in fact, that each ciliated zoospore is surrounded with a gelatinous or semi-fluid areola, of the same nature precisely as that which surrounds the embryo *Volvox* while within the parent, and in which also it is not I think possible to detect a distinct limitary membrane. This envelope of the ciliated zoospores contains a nitrogenous element, which sometimes, on the addition of iodine, gives rise to the appearance of minute beads around the outer periphery of each gelatinous mass, or in the lines of the hexagonal areas as seen in fig. 25. It is to be observed also, that connecting filaments of protoplasma may occasionally be seen to pass from one zoospore to another across the line of junction of the two gelatinous envelopes (fig. 24). These zoospores therefore of *Volvox* would appear to represent the "encysted zoospore" of Cohn (*Protococcus pluvialis*, &c.), and his fig. 43, plate 67, may perhaps be taken as a fair representation of what I conceive to be the condition in these connected zoospores in *Volvox*. This explanation of the hexagonal areolation, however, does not clash at all with that which I have given as to the structure of the wall of *Volvox*. For in this case, as in all others, the collected mass of zoospores, and their envelopes, is enclosed by a continuous external membrane, not in any way derived from them but from the parent cell in which they were originally formed.

There are several other interesting points relating to *Volvox* which have come under my observation in a prolonged attention to the subject, including another form of development of the internal spore, in which it divides, not in the usual way, into what may perhaps not inappropriately be considered as macrogonidia, to use Braun's expression, but into a much smaller and differently arranged sort, which may be considered as his microgonidia; but to enter fully upon this and other points would demand more space than is here at command.

[Whilst this paper is passing through the press, I have found that a faint, but quite distinct, purplish blue tinge may be produced in the wall of *Volvox globator* by means of Schultz's solution. The specimens of *Volvox* in which I have noticed this have been preserved in glycerine for two months.—G. B.]

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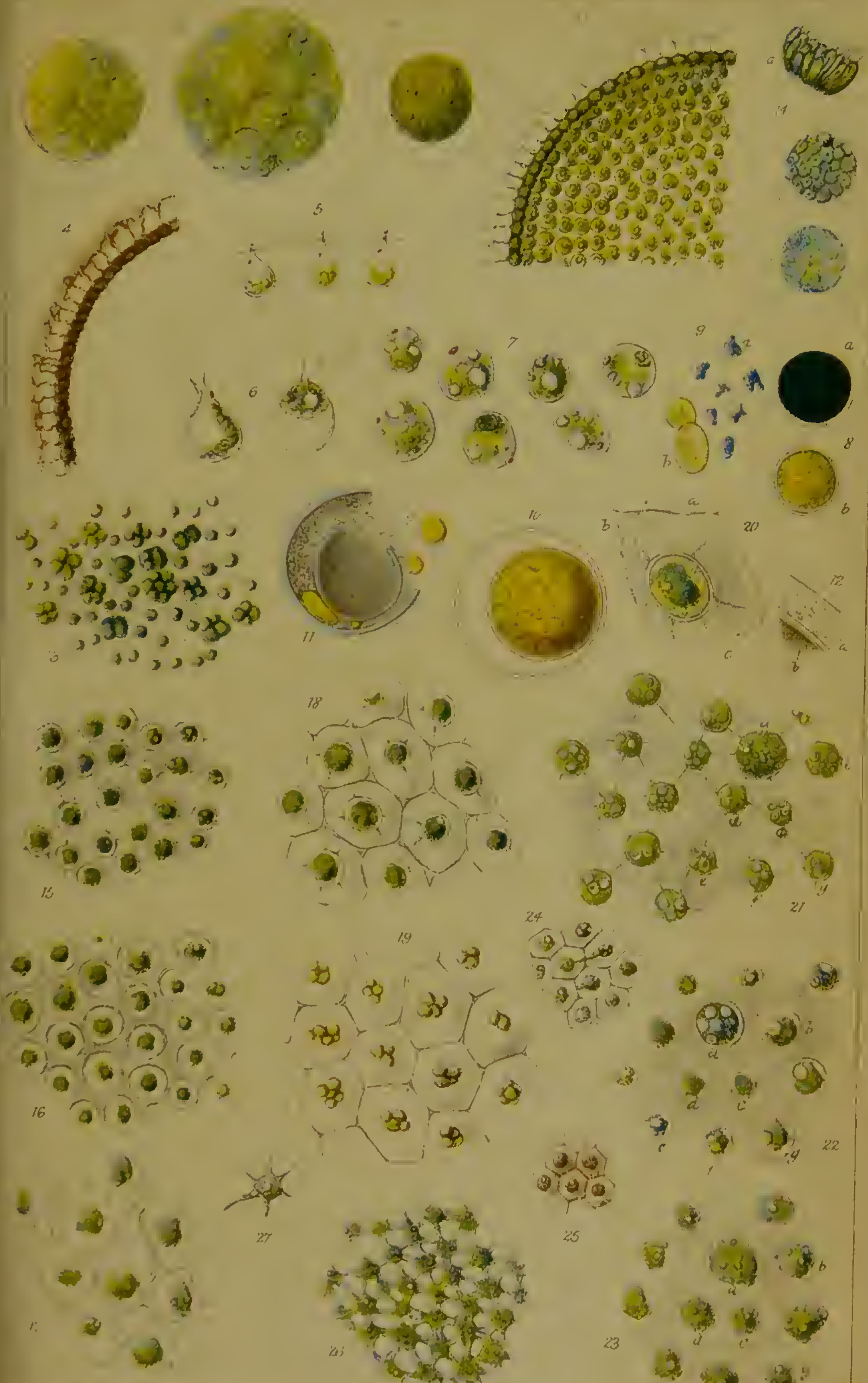

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